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ORIGINAL ARTICLE/ARTICOLO ORIGINALE

Properties of a novel polydimethylsiloxane endodontic sealer☆

Proprietà di un nuovo cemento endodontico a base di polidimetilsilossano

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KEYWORDS
Ion release;
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Wide apex;
Wet apex;
Guttaflow Bioseal.

Abstract
Aim: The study aims to assess sealing ability of a novel polydimethylsiloxane-based sealer in simulated wet root canals with wide apex, ion release (calcium and pH) and examine samples using ESEM.
Materials and methods: GuttaFlow bioseal, GuttaFlow2, and RoekoSeal Automix (Coltène/Whaledent Inc.) were tested.

Roots were prepared to obtain an apical diameter #40 using nickel–titanium rotary files (HyFlex CM, Coltène/Whaledent Inc.), each root was filled with single cone technique and immediately inserted into a simulated socket (filled with 0.02 mL of simulated body fluid) to reproduce the clinical conditions of a wet apical environment. Sealing ability was evaluated as fluid filtration rate at 1, 14, 28 days, and 10 months.

After 28 days in simulated body fluid, apices were examined using an Environmental Scanning Electron Microscope (ESEM).

Alkalinizing activity and calcium release was evaluated after 3 h and 1, 7, 14, and 28 days.

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Data were analysed using Kolmogorov–Smirnov test ($p < 0.05$).

Results: Fluid filtration analysis showed no significant difference within materials. Significant difference was observed between 10 months observation and other time frames ($p < 0.05$) of the same group.

GuttaFlow bioseal showed a significantly higher alkalinising activity ($p < 0.05$). Calcium release ability showed no significant difference through time, however significant differences were observed among materials ($p < 0.05$).

Observation using ESEM at 28 days after root obturation showed the presence of the materials sealing the wide apical foramen.

Conclusions: All materials showed satisfying sealing ability. However due to low calcium release, their use is not suggested when apical barrier formation and periapical bone regeneration are needed.

PAROLE CHIAVE
Rilascio ionico;
Polidimetilsilossano;
Capacità di sigillo;
Apici beanti;
Guttaflow Bioseal.

Riassunto

Obiettivi: Valutare il sigillo di un nuovo cemento endodontico a base di polidimetilsilossano in apici beanti-umidi tramite il metodo fluid-filtration, l'osservazione microscopica (ESEM), ed il rilascio ionico (calcio-pH).

Materiali e metodi: Sono stati testati i seguenti materiali: GuttaFlow bioseal, GuttaFlow2, e RoekoSeal Automix.

I campioni radicolari sono stati preparati con strumenti al nickel–titanio (HyFlexCM) fino ad un diametro apicale #40. Al fine di riprodurre le condizioni cliniche di umidità, a seguito dell'otturazione canalare con tecnica del cono singolo, i campioni sono stati inseriti in un alveolo artificiale (riempito con 0.02 mL di soluzione salina). La capacità di sigillo è stata valutata tramite fluid filtration analysis a 1, 14, 28 giorni e 10 mesi.

Dopo 28 giorni in soluzione salina, l'apice dei campioni è stato esaminato avvalendosi del Microscopio a Scansione Elettronica (ESEM).

Rilascio di calcio e pH sono stati valutati dopo 3ore e 1, 7, 14, 28 giorni.

I dati sono stati analizzanti usando il test Kolmogorov–Smirnov ($p < 0.05$).

Risultati: L'analisi del sigillo apicale ha mostrato differenze significative tra 10 mesi ed il restante dei tempi d'analisi ($p < 0.05$), mentre non ci sono state differenze tra i materiali.

GuttaFlow bioseal ha mostrato attività alcalinizzante significativamente più alta ($p < 0.05$). Ci sono state differenze statisticamente significative nel rilascio di calcio tra i materiali ($p < 0.05$), ma non tra i tempi.

L'analisi microscopica a 28 giorni dall'otturazione canalare ha confermato l'otturazione apicale da parte dei materiali.

Conclusioni: Tutti i materiali testati hanno mostrato capacità di sigillo soddisfacenti. Tuttavia a causa del ridotto rilascio di calcio riscontratosi, il loro utilizzo non è consigliato nei casi dove sia richiesta la formazione di una barriera apicale e rigenerazione ossea periapicale.

Introduction

A wide apex is a frequent manifestation of treatment failures or incomplete root development caused by traumatic events or carious lesions. In such cases, moisture management and sealing ability becomes challenging and no endodontic sealers is able to ensure a satisfying apical seal. This leads to an increasing interest of clinicians in materials able to seal in moist environment (water, blood or other fluids) and induce apical barrier formation when needed.

In treating teeth with wide and wet apices, polydimethylsiloxane (silicon)-based sealers may represent a viable alternative to calcium silicate-based sealer. They appear to have clinical benefits in terms of homogeneity and adaptation,[1] and absorb stress generated by mastication during root flexure due to their viscosity and elasticity.[2] Such materials are currently being marketed by Coltene Whaledent, as: RoekoSeal Automix, GuttaFlow 2, and GuttaFlow bioseal. RoekoSeal® Automix is one of the first silicon-based sealer productions of Coltene Whaledent, showing satisfying in vitro sealing ability up to 1 year in wide and straight canals.[3] GuttaFlow® 2 is an advancement of the existing GuttaFlow® (Coltene Whaledent) material in capsules; appearing to show a slight expansion during setting[4] and guaranteeing a tight seal.[5] According to in vitro studies, apical seal is either significantly improved[6] or similar when compared to AH Plus.[7] GuttaFlow® bioseal is Coltene's latest hydrophilic sealer containing gutta-percha powder, polydimethylsiloxane, and bioactive glass ceramic. GuttaFlow bioseal showed alkalinizing activity together with negligible solubility and slight

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calcium release when compared to GuttaFlow 2 and RoekoSeal Automix.[8] According to Gandolfi et al.,[8] the incorporation of a calcium silicate component may represent an attractive strategy to obtain a bioactive biointeractive flowable guttapercha sealer for moist/bleeding apices with bone defects.

The aim of the present study was to assess the sealing ability in simulated wide wet apices and ion release activity (pH of soaking water and calcium release) of polydimethylsiloxane-based sealers. Also examining root apex using environmental scanning electron microscopy.

Materials and methods

Materials

A comparison between the following endodontic sealers was undergone: polydimethylsiloxane-based sealers (Coltène/Whaledent Inc., USA) GuttaFlow bioseal, GuttaFlow 2, and RoekoSeal Automix (Table 1).

Sealing ability

Root preparation

Human caries free single-rooted extracted teeth ($N = 36$) with oval root canals were cleaned before preservation in a distilled water solution at 4 °C for less than a month. All teeth were sectioned at 12 ± 1 mm from the apex with a water-cooled diamond bur (FG Intensive n.D2 Lugano-Grancia, Switzerland), followed by thorough examination under an operating microscope (OPMI pico Zeiss microscope, Carl Zeiss S.p.A. Milano MI, Italy) in order to confirm the presence of a single oval-shaped canal. After working length establishment at the anatomic apex, canal shaping and debridement was performed with 0.02 taper stainless steel K-files and 0.04 taper nickel–titanium rotary instrument (HyFlex CM 40/04, Coltène/Whaledent GmbH + Co. KG, Langenau, Germany) using a micro-motor (X-SMART plus, Denstply, Maillefer Instruments Holding S.à.r.l., Switzerland). A step-down technique was performed until a size 40 apical sit was established (Fig 1). The canals were irrigated between each instrument with 0.5 mL of ethylenediaminetetraacetic acid (EDTA, Ogna, Muggiò, Italy) and 1 mL of 5% NaOCl (Niclor, Ogna, Muggiò, Italy). Both solution were delivered from a 30-G side-vented syringes inserted to 1 mm short of the working length, afterwards rinsed with deionized water for 1 min and dried with sterile paper points (Mynol, Milwaukee, WI, USA).

1.1.1. Root obturation and simulation of humid environment "Artificial alveolar chamber"

In order to reproduce the clinical conditions of a wet environment and apical stop, a customised wet chamber device was designed. A silicon-made support (Optosil; Heraeus Kulzer, Hanau, Germany) was specifically designed in order to create an artificial alveolus (Fig 2).[9] A simulated body fluid solution (0.02 mL of Hank's Balanced Salt Solution [HBSS]; Lonza, Verviers, Belgium) was added to 1/3 of the bottom of the support to simulate the presence of periapical fluid; the composition of the HBSS was (g/L) 0.4 KCl, 0.06 KH_2PO_4 anhydrous, 0.35 $NaHCO_3$, 8.0 NaCl, 0.05 Na_2HPO_4 anhydrous and 1.0 D-glucose.

Each root was placed inside the chamber so the periapical space and the entire root canal were filled by HBSS. The presence of fluid solution inside the root canal was assessed using paper points. The apical stop (i.e., silicon support) was 1 mm after apical foramen.

The six aforementioned endodontic sealers ($n = 6$ per group) were used for obturation. In all cases the obturation was followed by the insertion of a 40/0.06 taper gutta-percha point (Hygienic, Coltène/Whaledent Inc.) using single cone technique, after apical sit establishment (Figs. 3).

1.1.2. Fluid filtration analysis

A 18-gauge needle inserted across a plexiglass support was introduced into the coronal third of the filled root (5 mm) and the coronal side of the root was fixed to the plexiglass support with cyanoacrylate (Rocket, Corona, CA, USA) (Fig. 4). The external surface of root was coated with nail varnish to seal the root surface except for the apical orifice (2 mm apical free from varnish).[10]

Table 1 Tested materials and components.

Materials	Manufacturer	Lot n° and expiration date (year–month)	Ingredients
RoekoSeal Automix	Coltène/Whaledent Inc., OH, USA	6211034 (2015–12)	Polydimethylsiloxane, silicone oil, paraffin-base oil, platinum catalyst, zirconium dioxide
GuttaFlow 2	Coltène/Whaledent Inc., OH, USA	G07095 (2016–12) F51370 (2015–11)	Gutta-percha powder, polydimethylsiloxane, platinum catalyst, zirconium dioxide, cro-silver (preservative), colouring.
GuttaFlow bioseal	Coltène/Whaledent Inc., OH, USA	140814P3EZB (2016–08)	Gutta-percha powder, polydimethylsiloxane, platinum catalyst, zirconium dioxide, silver (preservative), colouring, bioactive glass ceramic

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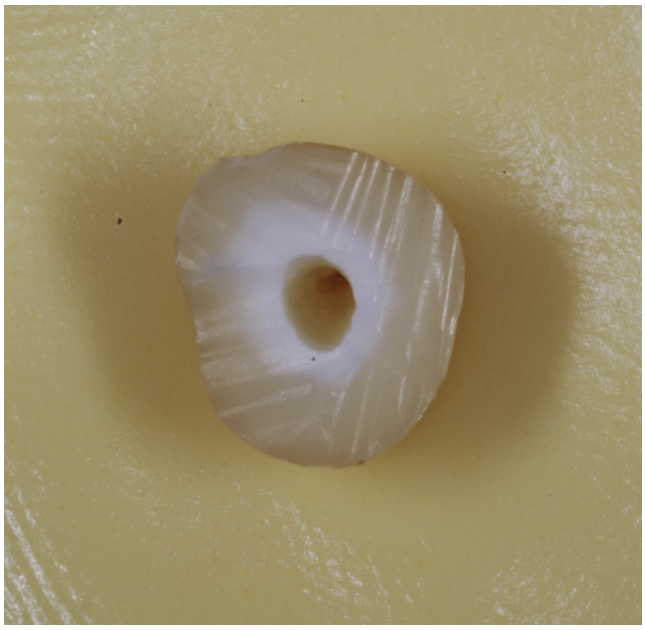

Figure 1 Sample preparation: before root obturation of samples.

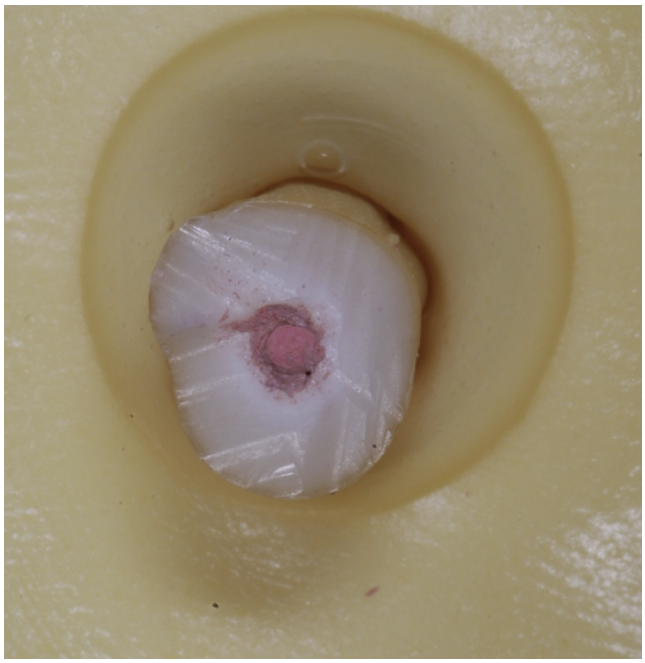

Figure 3 Sample preparation: after root obturation of samples.

A high-precision device (digital fluid flow meter) able to detect micrometric fluid movements through the apical system designed by Gandolfi was used for fluid flow rate measurements (Fig. 5). The micrometric forward movement of an air bubble inside the micro capillary was converted into a fluid flow measurement (microinfiltration) through the filled root. Each sample was connected to the device (through the 18-gauge needle) working at a hydraulic pressure of 6.9 kPa (1 psi) and contained deionised water with chlorhexidine 0.03% to prevent bacterial contamination inside the system.

The fluid filtration rate was measured (Fig. 6) after different storage of the sample in HBSS at 37 °C after 1, 14 and 28 days. Three measurements, of 4 min each, were made for individual samples, and the mean was calculated. The results

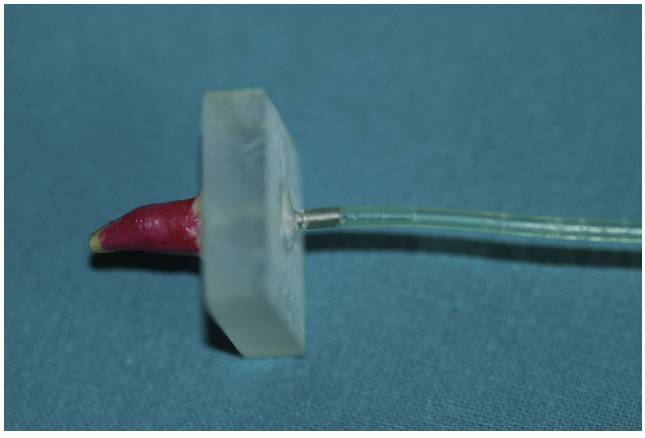

Figure 4 Before fluid filtration analysis: coronal side of root fixed to plexiglass support with cyanoacrylate.

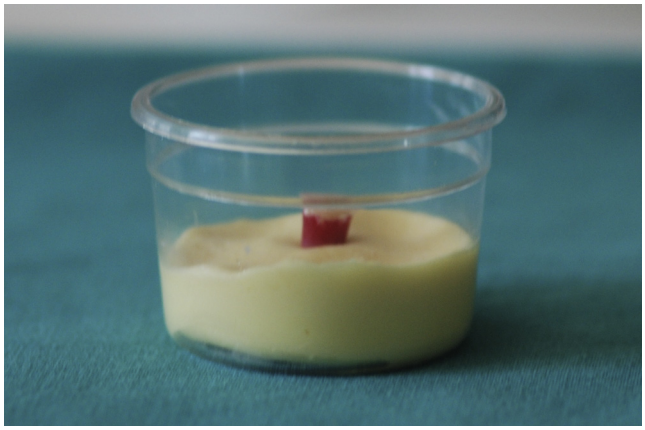

Figure 2 Wet chamber: sample are positioned in a customised silicon wet chamber.

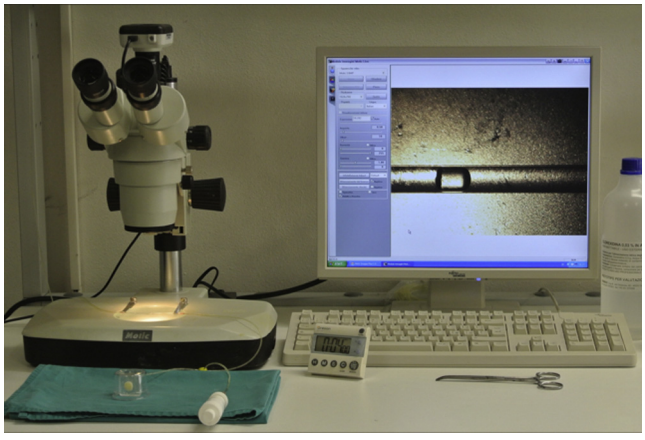

Figure 5 Digital fluid flow meter.

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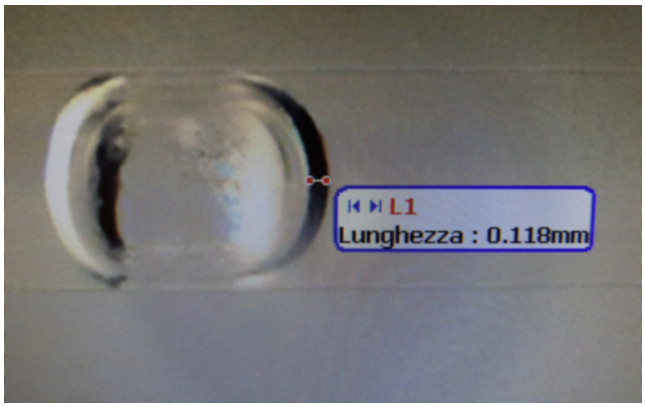


Figure 6 Measurements: Micrometric forward movement of the air bubble have been converted into micrometric fluid flow to evaluate the sealing ability.

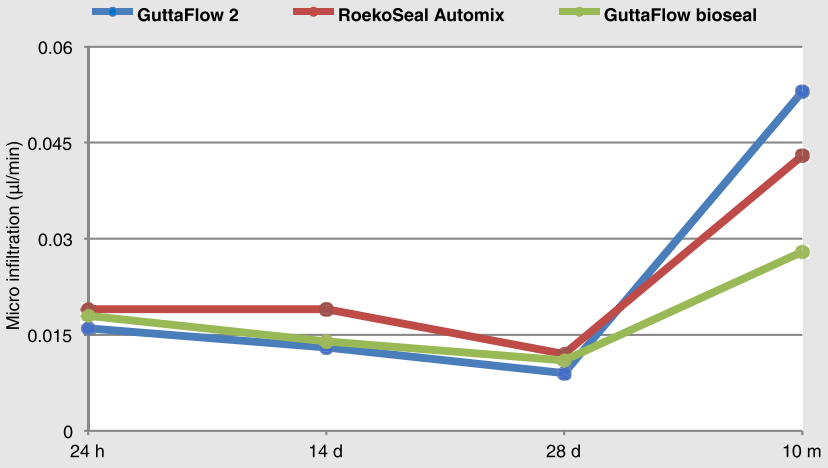
obtained as micrometers per minute were converted to μl/min.

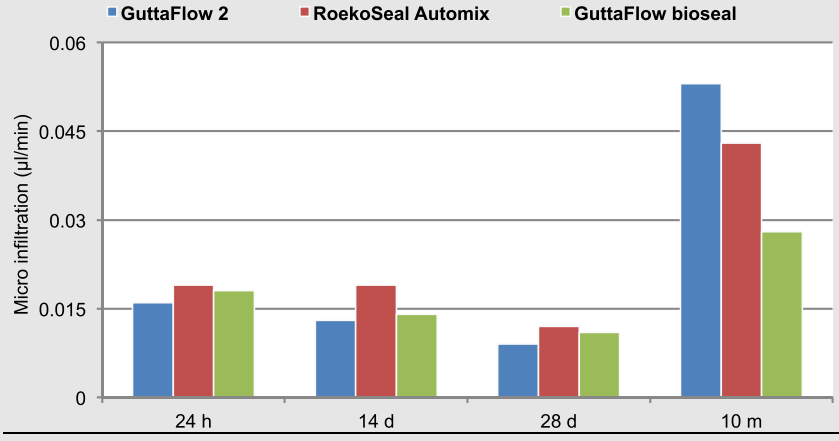
Calcium release and alkalinising activity (pH of soaking water)

Measurements were made starting from the compaction of freshly mixed sealers into PVC (polyvinyl chloride) moulds (8.0 ± 0.1 mm diameter and 1.6 ± 0.1 mm thickness), to prepare material disks ($n = 10$ for each material). The excess was removed leaving an exposed surface area on each sample of 50.24 ± 0.01 mm^2. Each mould was afterwards positioned inside a cylindric polypropylene container with 3 cm height and 4 cm diameter, filled with 10 mL deionised water (pH 6.8), sealed with its appropriate stopple, and stored at 37 °C. After 3 h, 1, 7,14, and 28 days, deionised water was collected for analysis and replaced. The collected water was analysed for pH and Ca release using a potentiometric method under

Table 2 Micro infiltration (μl/min, $n = 6$ per group) of filled root canals. Data with different superscript small letters in column (among materials) and superscript capital letters in row (within materials) are statistically different.

	24 hours	14 days	28 days	10 months
GuttaFlow 2	0.016 ± 0.010^{Aa}	0.013 ± 0.006^{Aa}	0.009 ± 0.002^{Aa}	0.053 ± 0.050^{Ba}
RoekoSeal Automix	0.019 ± 0.016^{Aa}	0.019 ± 0.008^{Aa}	0.012 ± 0.006^{Aa}	0.043 ± 0.030^{Ba}
GuttaFlow bioseal	0.018 ± 0.008^{Aa}	0.014 ± 0.010^{Aa}	0.011 ± 0.003^{Aa}	0.028 ± 0.025^{Ba}





magnetic stirring at room temperature (24 °C). Calculation was performed until the measurement stabilised.[11,12]

The pH was measured using a selective temperature-compensated electrode (Sen Tix Sur WTW, Weilheim, Germany) connected to a multi-parameter laboratory meter (inoLab 750 WTW, Weilheim, Germany) previously calibrated with standard solutions. The amount of calcium ions was measured using a calcium probe (Calcium ion electrode, Eutech instruments Pte Ltd, Singapore) after addition of 0.200 mL (2%) of ionic strength adjuster (ISA, 4 mol/L KCl, WTW, Weilheim, Germany). Then the mean and standard deviations were calculated.

Environmental Scanning Electron Microscope (ESEM) analysis

Root samples were prepared and obturated using sealing ability's methodology. In order to simulate a humid environment, samples were then completely immersed in simulated body fluid (HBSS) and stored at 37 °C for 28 days. Afterwards, apices were examined using an Environmental Scanning Electron Microscope (ESEM).[13]

All samples were examined uncoated at low vacuum (100 Pa), accelerating voltage of 20 kV, 8.5 mm working distance, 0.5 wt% detection level, 133 eV resolution, 100 μs amplification time. Sample apices were observed by ESEM at different magnifications ranging from 92× to 3000×.

Statistical analysis

The results were statistically analyzed. Kolmogorov–Smirnov test ($p < 0.05$) for fluid filtration, alkalinising activity and calcium release was performed.

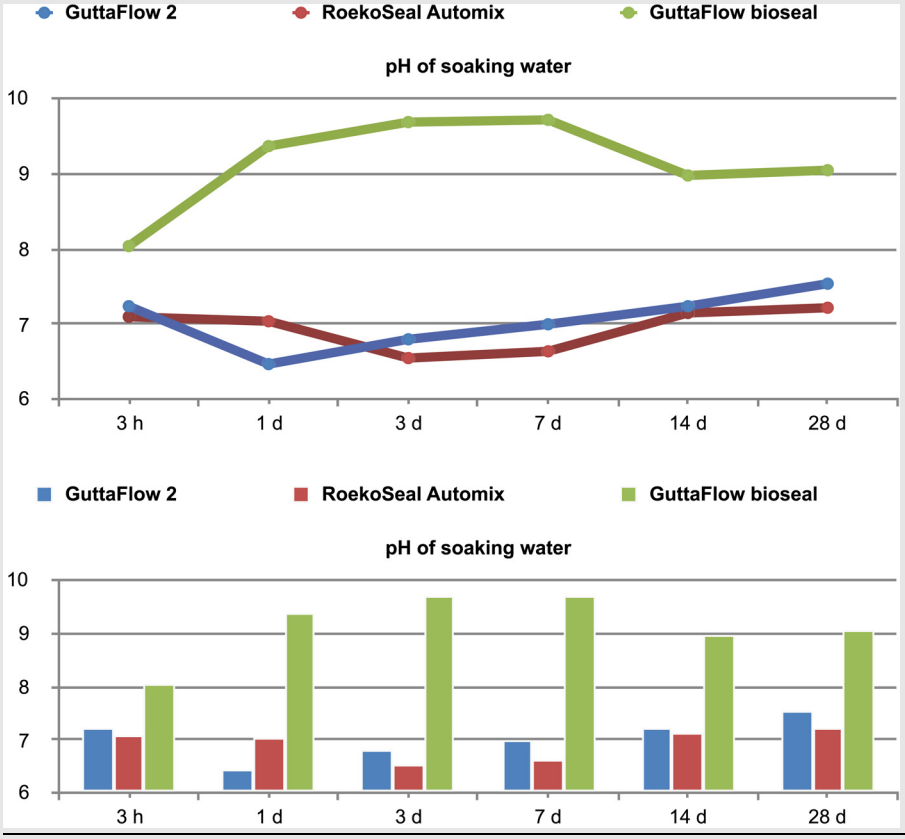
Results

Sealing ability

Table 2 reports the mean values of the fluid filtration rate (μl/min) of all groups at different storage times (1, 14, 28 days, and 10 months). No statistically significant difference was observed among the materials, while significant differences were observed when comparing 10 months observation with other time frames ($p < 0.05$). Even though at 10 months

Table 3 pH of soaking water ($n = 10$ for each material). Data followed by different superscript small letters in column (among materials) and superscript capital letters in row (within materials) are statistically different. Equal capital letters denote no statistical differences.

	3 h	1 day	3 days	7 days	14 days	28 days
GuttaFlow 2	7.24 ± 0.05^{Aa}	6.47 ± 0.08^{Aa}	6.80 ± 0.06^{Aa}	7.00 ± 0.09^{Aa}	7.24 ± 0.05^{Aa}	7.54 ± 0.08^{Aa}
RoekoSeal Automix	7.10 ± 0.09^{Aa}	7.04 ± 0.09^{Aa}	6.55 ± 0.08^{Aa}	6.64 ± 0.07^{Aa}	7.15 ± 0.10^{Aa}	7.22 ± 0.08^{Aa}
GuttaFlow bioseal	8.04 ± 0.15^{Ab}	9.37 ± 0.10^{Ab}	9.69 ± 0.07^{Ab}	9.72 ± 0.16^{Ab}	8.98 ± 0.02^{Ab}	9.05 ± 0.10^{Ab}



minimum flow of 0.028 ± 0.025 (GuttaFlow bioseal) and maximum of 0.053 ± 0.050 (GuttaFlow 2) were recorded, no significant difference was observed.

Calcium release and alkalinising activity (pH of soaking water)

Statistically significant differences were observed among materials. GuttaFlow bioseal showed a significantly higher alkalinising activity ($p < 0.05$). While no significant differences were observed among materials through time (Table 3).

Calcium release ability showed no significant difference through time, however significant differences were observed among all three materials ($p < 0.05$) (Table 4).

Environmental scanning electron microscope (ESEM) analysis

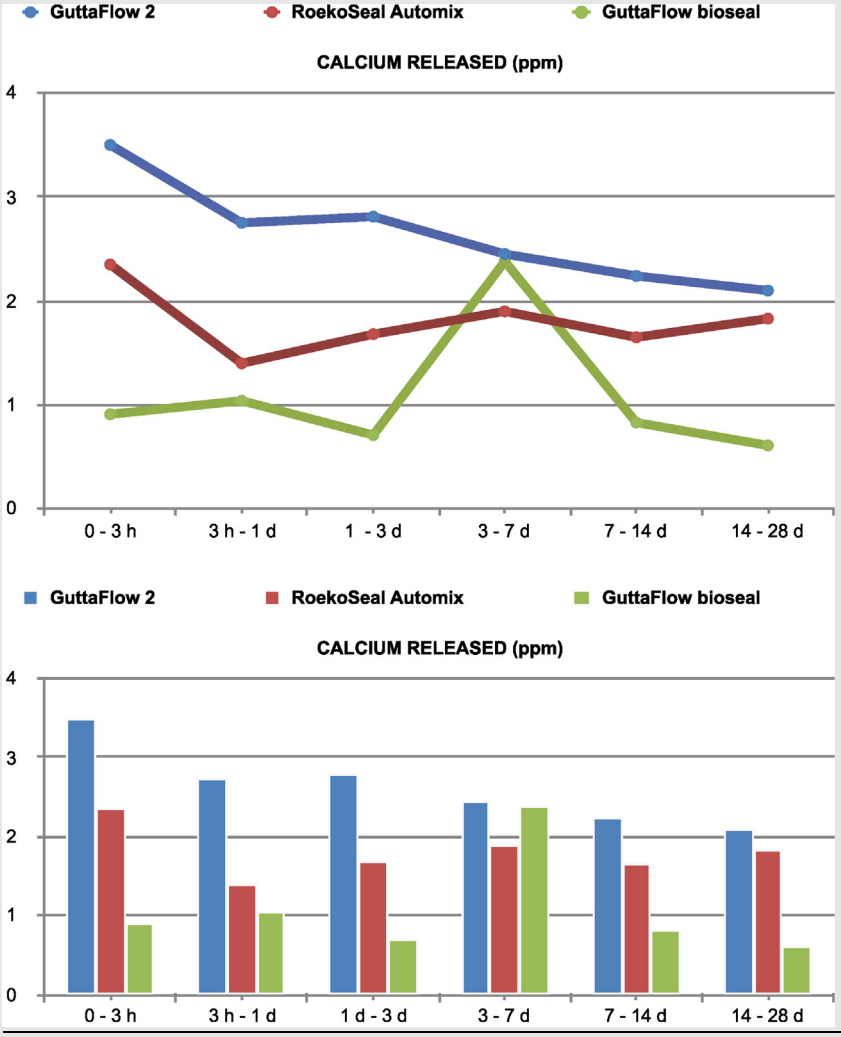
Fig. 7 reports ESEM observations of the apical region after 28 days of root obturation and immersion in simulated body fluid (HBSS). All images show the presence of the materials sealing the wide apical foramen. Higher magnification indicates morphological differences.

Discussion

Being able to estimate the material's sealing ability degradation process overtime gives the opportunity to have an insight on the severity of microbial apical leakage. Most studies conducted on sealing ability are unable to provide such

Table 4 Calcium released in ppm ($n = 10$ for each material) in 10 mL of deionized soaking water at 37 °C. Data followed by different superscript small letters in column (among materials) and superscript capital letters in row (within materials) are statistically different.

	0—3 hours	3 hours—1 day	1—3 days	3—7 days	7—14 days	14—28 days	Cumulative
GuttaFlow 2	3.50 ± 0.25^{Aa}	2.75 ± 0.41^{Aa}	2.81 ± 0.28^{Aa}	2.45 ± 0.29^{Aa}	2.24 ± 0.28^{Aa}	2.10 ± 0.29^{Aa}	$15,86 \pm 1.89^{a}$
RoekoSeal Automix	2.35 ± 0.11^{Ab}	1.40 ± 0.11^{Ab}	1.68 ± 0.13^{Ab}	1.90 ± 0.19^{Ab}	1.65 ± 0.17^{Ab}	1.83 ± 0.17^{Ab}	$19,81 \pm 1.11^{b}$
GuttaFlow bioseal	0.91 ± 0.09^{Ac}	1.04 ± 0.10^{Ac}	0.71 ± 0.04^{Ac}	2.38 ± 0.44^{Ac}	0.83 ± 0.09^{Ac}	0.61 ± 0.09^{Ac}	$6,46 \pm 1.57^{c}$



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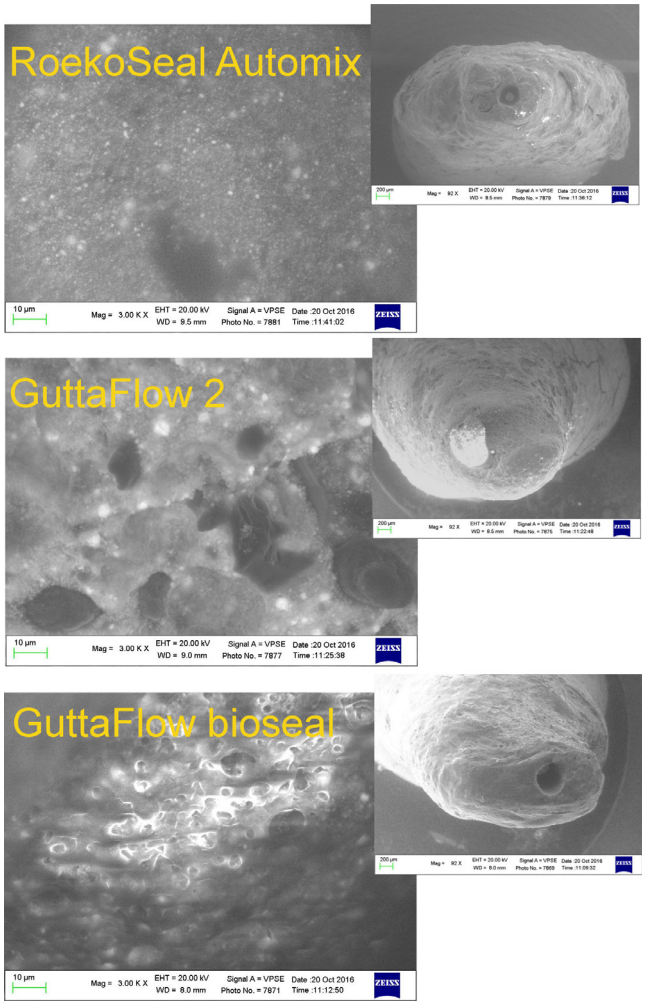

Figure 7 ESEM images showing wide apices of the roots filled with the tested materials at magnification ranging from 92× to 3000×.

information due to the fact that observations are generally restricted to 3 months. A 10-month follow-up is needed to presumably collect reliable data.

Even though fluid filtration test in wide and wet apices at 10-month follow-up showed no statistically significant difference among materials, bioglass-based GuttaFlow bioseal recorded the lowest values of apical leakage and GuttaFlow 2 the highest. Significant differences were observed at 10 months observation, possibly due to the sealer's degradation process overtime.

GuttaFlow bioseal showed satisfying alkalinizing activity, being in accordance with recent findings.[8] In this study the highest pH value ranging from 8 to 9 of GuttaFlow bioseal, suggests antibacterial activity which could be related to its bioactive glass composition. Increase in hydroxyl ions leads to low concentrations of bacteria such as the *Enterococcus faecalis*, a major responsible for root canal treatment failures.[14]

According to a study[15] on the effect of pH and ionic strength on the reactivity of bioglass, only at pH 8 a total reconstruction of glass occurs, comprising silica and calcium phosphate rich layers formation. At higher pH (pH > 9), selective dissolution is impeded by an immediate precipitation of a calcium phosphate layer, meanwhile at lower pH a total breakdown of glass is observed. Calcium release is observed to be slower in water, probably because of the faster formation of calcium containing salts, which are less soluble at higher pH.

When immersed in a simulated body fluid, the calcium ions released with phosphate promote the formation of a superficial layer of calcium phosphate (CaP) able to fill the open voids. On a clinical standpoint, the combination of the apatite (CaP) forming ability[16,17] and gutta-percha could result in an improvement of the sealer's sealing ability[18] due to CaP deposition.[13]

The sealing ability evaluated showed a stable behaviour up to 28 days. However, at 10 months evaluation a reduction was observed. Such significant difference might be due to the intrinsic degradation process of the materials. Even though, no statistical difference was observed at 10-month follow-up among materials, GuttaFlow bioseal's lower flow values may indicate calcium phosphate deposition,[8] and therefore better sealing ability.

ESEM analysis at 28 days after root obturation gave insights on the wide apical sealing quality in wet environment.

Conclusions

All sealers showed adequate sealing abilities with no statistically significant difference among materials. Significant differences were observed when fluid filtration test was prolonged for 10 months, suggesting that a higher apical leakage could be related to the sealer's degradation process overtime.

Clinical relevance

GuttaFlow bioseal demonstrated to be a promising endodontic material used in teeth with wide (apical diameter 40) and wet apices. Nonetheless, low calcium release evaluated limits its adoption in conditions were an apical barrier formation is required.

Conflict of interest

The authors deny any conflict of interest related to this study.

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